

CONSTRUCTION OF UNI-DIRECTIONALLY CLONED cDNA LIBRARIES FROM
MESSENGER RNA FOR IMPROVED 3' END DNA SEQUENCING

ABSTRACT

Methods are provide for preparing cDNA corresponding to a mRNA. In the subject methods, a mRNA is first contacted with a mixture of primers under first strand cDNA synthesis conditions. The primer mixture contains primers that have at least 10 contiguous deoxythymidines, a double stranded restriction enzyme recognition sequence near one end and a non-polyA-complementary region near the other end, where the non-polyA-complementary region is -VV, -VTV, -VTTV, -VTTTV, and -VVVVV. The resultant cDNA is modified such that the polyT tail is substantially removed. The modified cDNA is then ligated into a vector. The subject methods find use in a variety of applications, and find particular use in the sequencing of DNA and in the synthesis of cDNA libraries.